Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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Zika virus disease and associated neurological disorders in Brazil

Supplementary Appendix

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Contents

1. Sentinel surveillance

In 2015, the Ministry of Health registered 150 sentinel units (SU, clinics and hospitals) in 23 of 27 UF (states), an average of 5.6 sentinel sites in each of the 27 states (database Sinan_net). The SU data base was created using the web-based system of forms known as FORMSUS.

The sentinel reporting sites were chosen to identify areas of possible transmission across the whole country. Case reports from these sites were not intended to measure the absolute size of the epidemic and burden of disease (they would have reported only a small fraction of symptomatic cases of Zika virus infection, and many would have been asymptomatic), but they may accurately reflect the comparative size and timing of the epidemic across states.

Of the 150 SU, 26 (17%) were from the North, 33 (22%) from the North-East, 48 (32%) of the South, 19 (13%) of the South-East and 30 (20%) of the Mid-West. Because the presence of Zika virus was confirmed only in May 2015, some sentinel sites may not have reported cases early in the year. Three states (Pernambuco, Bahia and Parana) carried out universal surveillance from all health facilities. The great majority of cases reported in Sinan_net during 2015 were from Bahia state. SUs followed MoH diagnostic and reporting criteria; other health facilities did not necessarily do so.

2. Case definitions

Zika virus disease

Case series for Zika virus disease in the main text include both suspected and confirmed cases reported from the sentinel surveillance system that was in operation during 2015.[1]

Suspected case: patient with pruritic maculopapular rash with two or more of the following signs and symptoms: fever, or conjunctival hyperemia without secretion and itching, or polyarthralgia, or periarticular edema.

Confirmed case: suspected case with one of the following positive tests: Specific reagents for diagnosis of Zika: virus isolation; detection of viral RNA by reverse transcriptase reaction (RT-PCR); (IgM) serology test.

Discarded case: a suspected case that has one or more of the following criteria:

- Nonreactive serology test (IgM), since the samples have been taken in a timely manner, packaged and transported properly;
- Diagnosis of another disease;
- Negative laboratory test (RT-PCR) or without laboratory examination, whose clinical and epidemiological background is consistent with other diseases.

Guillain-Barré syndrome

The data used by the Dengue-Chikungunya-Zika National Program consists of notifications from all public and private health units to SUS (universal coverage health public program) via Sinan_net. We assume that almost all GBS cases present at a hospital, especially a public hospital because of the high cost of the treatment and monitoring. Physicians from those hospitals make a GBS diagnosis, which is used for the SIH (hospital database) registry and coded according to ICD-10. This source of information is a system used for making payments for medical procedures. Under-reporting is unlikely, at least from the public sector, because GBS is a high-cost procedure and a serious disease. However, some cases reported as GBS could be due to other severe neurological disorders.

[1] Source: Nota Informativa - SVS/MS, Ministério da Saúde, Secretaria de Vigilância Em Saúde.

Since December 2015, the protocol[2] for surveillance of cases with neurological events, with history of previous viral infection, has used the following definitions:

Suspected case: patient with neurological manifestation of undetermined origin who attended a sentinel unit (SU), and with a previous viral infection up to 60 days before the onset of neurological symptoms. Neurological manifestations include: encephalitis, meningoencephalitis, myelitis, acute flaccid paralysis, acute disseminated encephalomyelitis (ADEM) and/or Guillain-Barre syndrome. Here the SUs are hospitals only, and not necessarily the same SUs as used for Zika case detection.

Probable case: suspected case with clinical signs of Zika virus infection, dengue or chikungunya, without laboratory confirmation.

Confirmed case: suspected case with laboratory confirmation by RT-PCR for the following etiological agents: Zika (samples of cerebrospinal fluid, urine or serum), Dengue (CSF or serum samples), Chikungunya (CSF or serum samples).

Discarded case: patient that was suitable as suspected case for other disease and: another etiological agent was confirmed such as: Epstein-Barr, herpesvirus, cytomegalovirus, Campylobacter, and others (excluding zika, chikungunya and dengue virus), OR another diagnosis was made by the physician, such as stroke, diabetic acidosis, among others.

Microcephaly

The definition of microcephaly due to suspected Zika virus infection has changed during 2015 and 2016. Since epidemiological week 44/2015, all term neonates (37 to 42 weeks of gestational age) with head circumference ≤ 33 cm should be reported to a surveillance database (both sexes). For pre-term neonates, reference should be made to the Fenton growth curve.

Between weeks 50/2015 and 10/2016, the cut-off point was changed to 32cm for term neonates (both sexes). For pre-term neonates, the Fenton curve still applied.
Since week 11 of 2016, the cut-off point has been <2 standard deviations (SD) according to the WHO reference for term neonates, and the intergrowth reference for pre-term

[2]Source: http://portalsaude.saude.gov.br/images/pdf/2016/fevereiro/05/Protocolo-de-vigilancia-de-manifestacoes-neurologicas.pdf

neonates. All cases are notified in the RESP database (Registro de Eventos em Saúde Pública - Event records in Public Health).[3]

Since 1996, SINASC (national database of births) has collected information on new-born children nationwide, and uses the ECLAMC (Latin American Collaborative Study of Congenital Malformations) definition of microcephaly, namely cephalic perimeter <3 SD, using WHO tables; given this restrictive definition, SINASC includes severe microcephaly cases only.

The present analysis of microcephaly cases uses RESP data only.

3. Excess cases of Guillain-Barré syndrome in 2015 and 2016

Figure 1B of the main text shows the aggregated weekly excess cases of GBS cases in 2015 and 2016. The weekly excesses in each of the years 2015 and 2016, as given in the main text, were calculated by subtracting from the averages for corresponding weeks in each of the 5 years 2010-14. The maximum numbers of GBS cases per week in the North-East region in 2015 and 2016 were more than twice the maximum in any preceding year (Figure S1). For all other regions, the peak in 2016 was 1.7 times higher than in any preceding year.

4. Time lag between GBS and microcephaly

The time lags between Zika virus disease and microcephaly and GBS and microcephaly (Figure 1B, main text) can be estimated from cross correlation of the case series – essentially, we vary the time lag to obtain the best correlation between two time series.

For the data in Figure 1B (North-East region), correlation coefficients are at a maximum at 22 weeks for GBS-microcephaly (Figure S2). The timing of GBS cases (routine hospital reports) is likely to be more accurate than the timing of cases of Zika virus disease (the sentinel surveillance system was being established during 2015). If the time delay from Zika infection to GBS was 3 weeks (as in French Polynesia), then Zika infections would have occurred 38 weeks (gestation) minus 23 weeks minus 3 weeks, or at 12 weeks post-conception, on average. This implies that about half the infections leading to microcephaly occurred during the first trimester of pregnancy (i.e. within 38/3 = 12.7 weeks).

[3]Source: http://portalsaude.saude.gov.br/images/pdf/2016/marco/24/Microcefalia-Protocolo-vigil--ncia-resposta-versao2.1.pdf

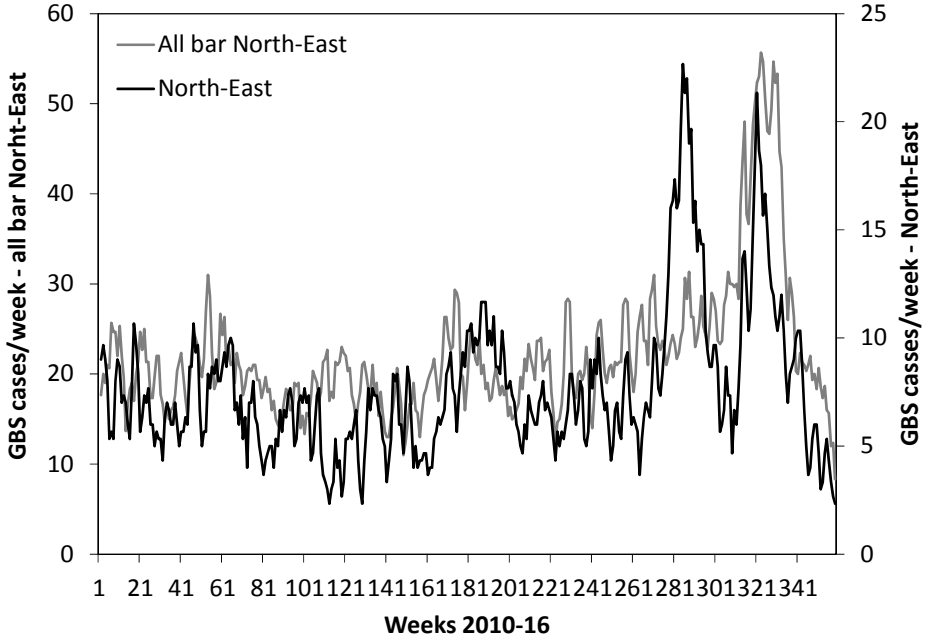

Figure S1. Cases of Guillain-Barré syndrome reported weekly from the North-East region (black), and from all regions except the North-East (grey). In the North-East there is a clear excess of cases in both 2015 and 2016; in other regions only in 2016. Data are plotted as 3-week moving averages. Source: Ministry of health, hospital data system (SIH).

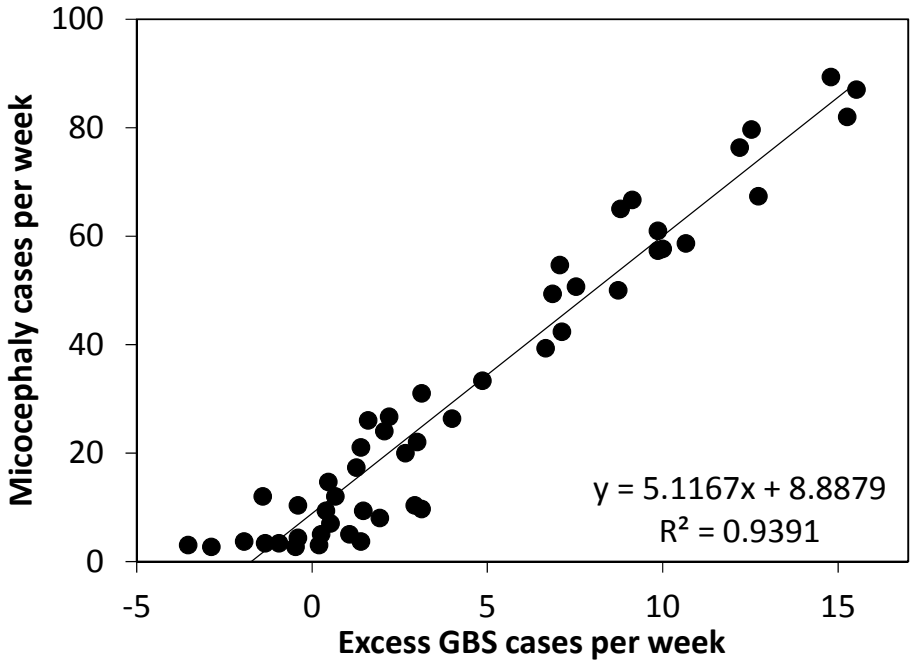

Figure S2. Cross-correlation of time series to identify delays between GBS and microcephaly. Cross-correlations are at a maximum at 23 weeks delay for GBS-microcephaly.

The proportion of infections that occurs in the first trimester depends on the time distribution of infections that lead to microcephaly e.g. for an overdispersed distribution, where the median < mean, a higher proportion of infections would occur earlier (and thus a higher proportion would occur in the first trimester). We cannot determine this distribution from the case series.

5. Reported cases of chikungunya, dengue and Zika in the North-East region, 2015-16

Chikungunya, dengue and Zika virus diseases present with similar symptoms – fever, muscle and joint pain, and skin rash – and all three viruses are transmitted by *Aedes aegypti* mosquitoes. Because cases of illnesses due to the three viruses coincide in space and time (Figure S3), there is a risk of misclassification, especially of Zika as Dengue in 2015 and of Chikungunya as Zika in 2016.

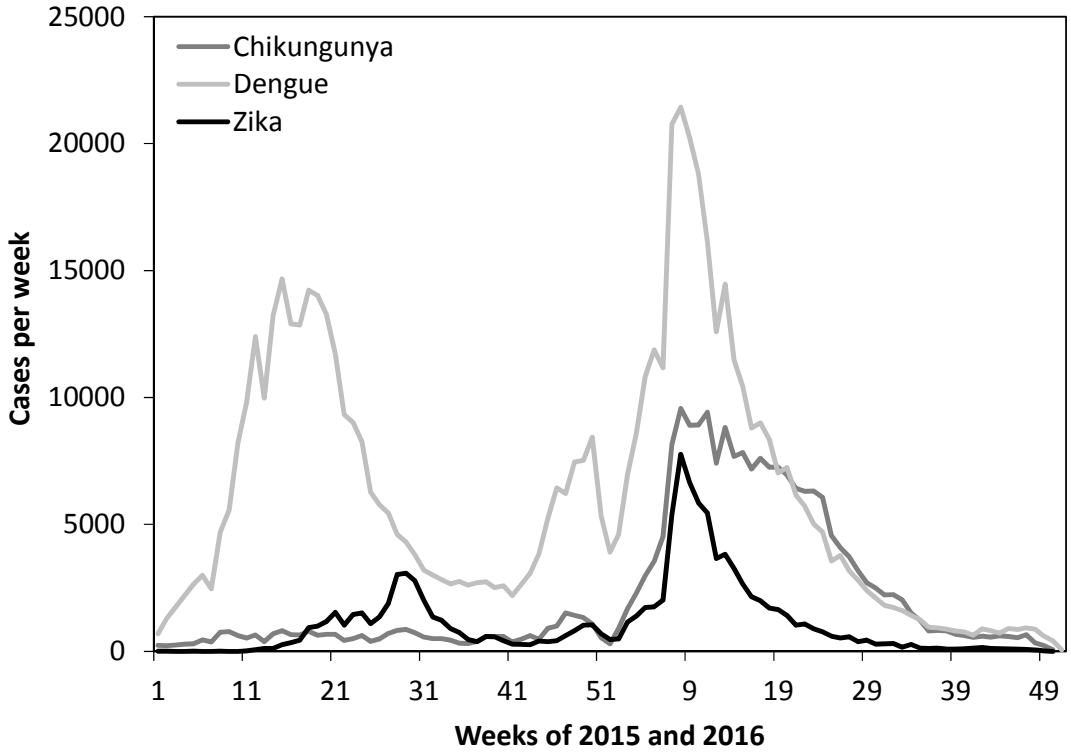

Figure S3. Co-existence in the North-East region of three arboviruses transmitted by *Aedes aegypti*. Data are plotted as 3-week moving averages.

6. Trends in birth and abortion rates in Brazil, 2015-16

The National data base of births and abortions reveals that both are strongly seasonal in Brazil, with births peaking in March and April each year (weeks 10-20 approximately) (Figure S4). Abortions precede births by 26 weeks on average (with much variation, Figure S5), from which we infer that the average time of abortion is 38 – 26 = 12 weeks of gestation, at the end of the first trimester. The threat of Zika virus might have affected abortions (greater in number) or births (fewer in number due to fewer conceptions or more abortions) in 2016. The routinely collected data presented in Figure S4 cannot be used to evaluate these possibilities because they are incomplete for 2016.

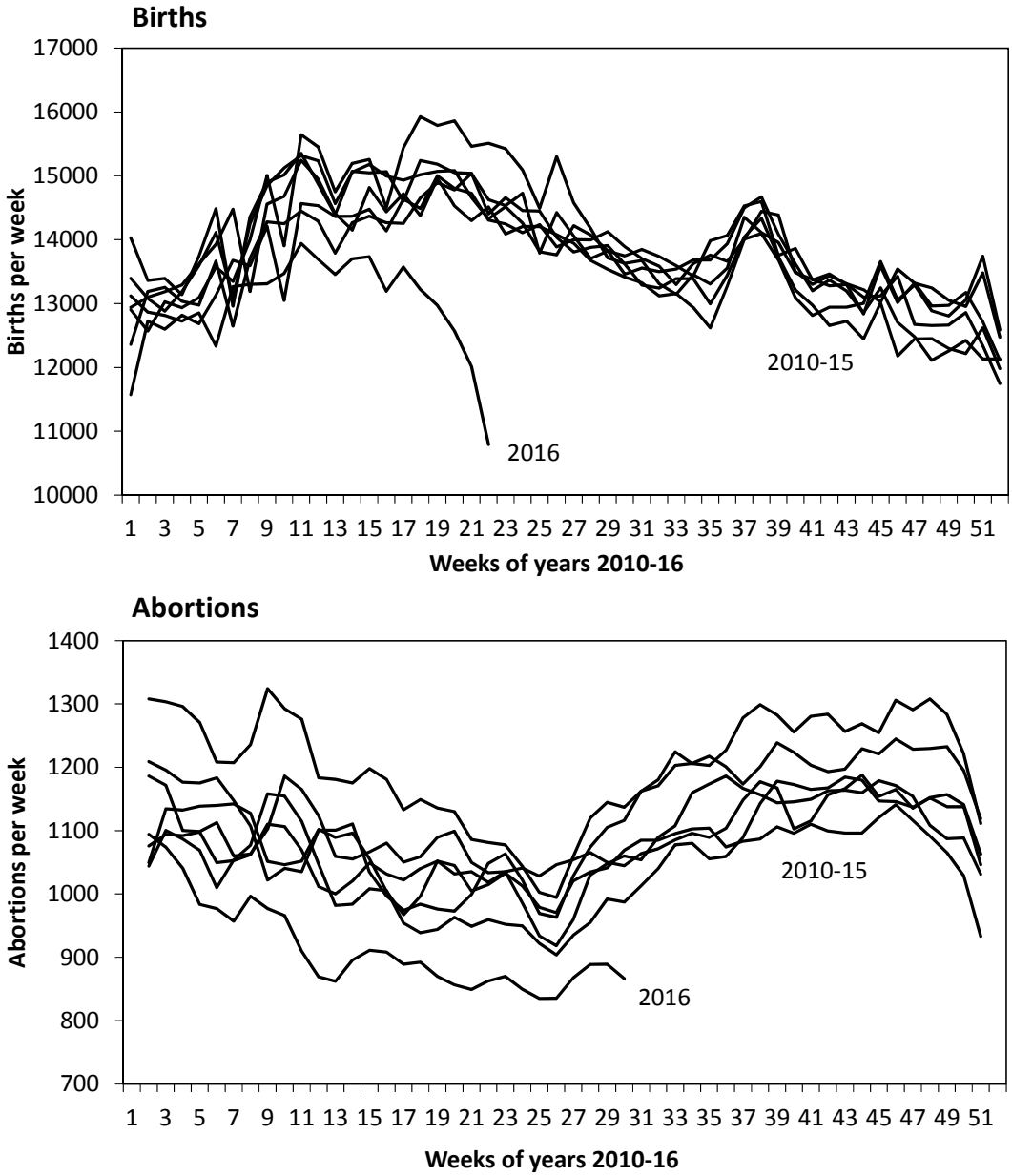

Figure S4. Weekly variation in births and abortions in the North-East region of Brazil, 2010-16.

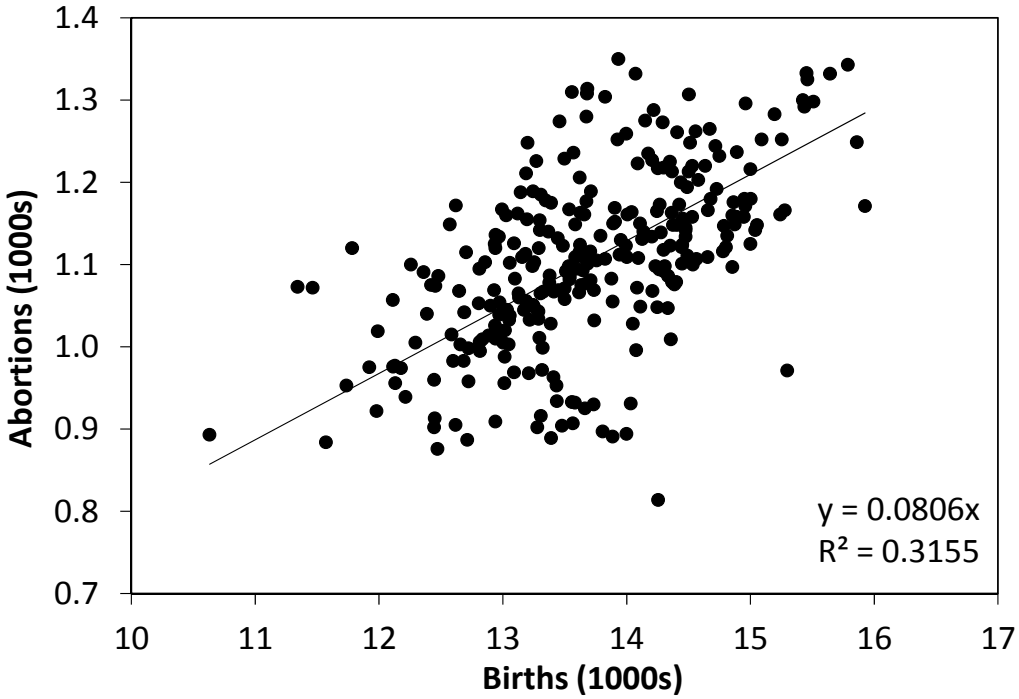

Figure S5. North-East region of Brazil: Abortions are 8.1% of births on average, and precede births by 26 weeks on average (cross-correlation analysis of time series). The scatter plot illustrates the variability in the data (r^2 max = 0.32 with a lag of 26 weeks).

7. Additional reading

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